Patent
Attorney Docket: 265/018

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of:	)	
	)	**Group Art Unit:** 1655
JOHN J. CARRINO et al.	)	
	)	**Examiner**: Taylor, J
Serial No.: TO BE ASSIGNED	)	
	)	
Filed: HEREWITH	)	
	)	
For: MULTIPLEX AMPLIFICATION AND SEPARATION OF NUCLEIC ACID SEQUENCES USING LIGATION-DEPENDANT STRAND DISPLACEMENT AMPLIFICATION AND BIOELECTRONIC CHIP TECHNOLOGY	)	

PRELIMINARY AMENDMENT

Attention: BOX PATENT APPLICATION
Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants herewith file a divisional application from pending USSN 09/290,577, filed April 12, 1999, which will issue May 29, 2001, as U.S. Patent No. 6,238,868. Please enter the following preliminary amendments before the calculation of fees:

IN THE CLAIMS:

OC-74764.1

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Please cancel claims 1-43.

Please add new claims 44-210:

44. A method for the amplification of at least one target nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

    a. contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

    b. ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

    c. using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, wherein each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template,

    wherein steps a, b, and c occur at the same time.

45. The method of claim 44 wherein a second SDA primer is utilized in addition to the first SDA primer in the strand displacement reaction.

46. The method of claim 45 wherein the first SDA primer is attached to a substrate.

47. The method of claim 46 wherein the first SDA primer is attached through a biotin/streptavidin interaction.

48. The method of claim 46 wherein the first SDA primer is attached through a covalent bond.

49. The method of claim 45 wherein the first and second SDA primers are individually attached to a substrate.

50. The method of claim 49 wherein the first and second SDA primers are attached through a biotin/streptavidin interaction.

51. The method of claim 49 wherein the first and second SDA primers are attached through a covalent bond.

52. The method of claim 44 wherein the first SDA primer is attached to a substrate.

53. The method of claim 52 wherein the first SDA primer is attached through a biotin/streptavidin interaction.

54. The method of claim 52 wherein the first SDA primer is attached through a covalent bond.

55. The method of claim 44 wherein the downstream ligation probe has a 3' terminus that is modified to be non-extendable by DNA polymerases.

56. The method of claim 44 wherein a plurality of target nucleic acid sequences are amplified within the same reaction mixture.

57. The method of claim 56 wherein the first SDA primer is a universal amplification primer capable of hybridizing to all ligated target probe templates.

58. The method of claim 56 wherein at least three target nucleic acids are amplified.

59. The method of claim 56 wherein at least four target nucleic acids are amplified.

60. The method of claim 44 wherein no bumper primers are used in the strand displacement amplification.

61. The method of claim 44 wherein amplicons of the ligated target probe template are detected.

62. The method of claim 61 wherein detection of amplicons is by at least one method selected from the group consisting of fluorescent detection, chemiluminescent detection, and electrochemiluminescent detection.

63. The method of claim 61 wherein the detection is by hybridization of a labeled oligonucleotide probe to the amplicon species.

64. The method of claim 63 wherein the hybridization probe is specific for an allele of a gene.

65. The method of claim 63, further comprising the step of thermally denaturing any double stranded amplicon species prior to detecting the amplicon species.

66. The method of claim 63,further comprising the step of electronically denaturing any double stranded amplicon species prior to detecting the amplicon species.

67. The method of claim 61 wherein the amplification step and detecting step occur simultaneously in relation to one another.

68. The method of claim 61 wherein the amplification step and detecting step occur consecutively in relation to one another.

69. The method of claim 61 wherein the detection is by the incorporation of a labeled nucleotide into the amplicon.

70. The method of claim 69 wherein the amplification step and detecting step occur simultaneously in relation to one another.

71. The method of claim 44 wherein the restriction endonuclease which recognizes the restriction endonuclease recognition sequence is BstBNI.

72. The method of claim 44 wherein the restriction endonuclease which recognizes the restriction endonuclease recognition sequence is selected from the group consisting of: HincII, HindII, Bso BI, AvaI, Fnu4HI, Tth111I, and NciI.

73. The method of claim 44 wherein the restriction endonuclease which recognizes the restriction endonuclease recognition sequence is selected from the group consisting of: BstXI, BsmI, BsrI, BsaI, NlaV, NspI, PflMI, HphI, AlwI, FokI, AccI, TthIII, Mra I, Mwo I, Bsr BI, Bst NI, Bst OI, and Bsm AI.

74. The method of claim 44 wherein in the strand displacement amplification step (c) at least one modified nucleic acid is incorporated into the amplicon, wherein the modification prevents cleavage of a polynucleotide containing the modified nucleic acid by restriction endonucleases.

75. The method of claim 74 wherein the modified nucleic acid is methylated.

76. The method of claim 74 wherein the modified nucleic acid is phosphorothiolated.

77. The method of claim 74 wherein the modified nucleic acid is selected from the group consisting of 2'deoxyadenosine 5'-O-(1-thiotriphosphate), 5-methyldeoxycytidine 5'-triphosphate, 2'-deoxyuridine 5'-triphosphate, and 7-deaza-2'-deoxyguanosine 5'-triphosphate.

78. The method of claim 45 wherein the strand displacement amplification in step (c) utilizes an unequal effective concentration ratio of the first SDA primer to the second SDA primer, wherein the first and second SDA primers form a set of primers, and wherein unequal populations of complementary first and second amplified strands of the target nucleic acids are formed in step (c).

79. The method of claim 78 wherein the unequal effective concentration ratio is obtained by providing at least one SDA primer of the set in molar excess as compared to the other SDA primer in the set.

80. The method of claim 79 wherein the SDA primer that is in molar excess is labeled.

81. The method of claim 80 wherein the label is a fluorescent label.

82. The method of claim 81 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

83. The method of claim 80 wherein the label is a chemiluminescence label.

84. The method of claim 80 wherein the label is an electrochemiluminescence label.

85. The method of claim 80 wherein the label is biotin.

86. The method of claim 78 wherein the unequal effective concentration ratio is obtained by providing a competitor in the amplification reaction for either the first SDA primer or the second SDA primer, and wherein the competitor is selected from the group consisting of: non-extendable competitors, non-cleavable competitors, and competitors which are non-extendable and non-cleavable.

87. The method of claim 86 wherein the competitor is anchored to a substrate.

88. The method of claim 86 wherein the competitor is in solution.

89. The method of claim 86 wherein the competitor is a non-extendable competitor.

90. The method of claim 89 wherein the competitor comprises a non-extendable 3' modification selected from the group consisting of: a 3' terminal base mis-match, a 3' dideoxy nucleic acid, and a blocking group attached to the 3' hydroxy group of the 3' terminal nucleic acid.

91. The method of claim 86 wherein the competitor is a non-cleavable competitor.

92. The method of claim 91 wherein the non-cleavable competitor comprises a nucleic acid selected from the group consisting of: methylated nucleic acids and phosphorothiolated nucleic acid.

93. The method of claim 91 wherein the non-cleavable competitor comprises a modified nucleic acid selected from the group consisting of 2'deoxyadenosine 5'-O-(1-thiotriphosphate), 5-methyldeoxycytidine 5'-triphosphate, 2'-deoxyuridine 5'-triphosphate, and 7-deaza-2'-deoxyguanosine 5'-triphosphate.

94. The method of claim 86 wherein the SDA primer which the competitor does not compete with is labeled.

95. The method of claim 94 wherein the label is a fluorescent label.

96. The method of claim 95 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

97. The method of claim 94 wherein the label is a chemiluminescence label.

98. The method of claim 94 wherein the label is an electrochemiluminescence label.

99. The method of claim 94 wherein the label is biotin.

100. The method of claim 44 wherein at least one of the first and second SDA primers is labeled.

101. The method of claim 100 wherein the label is a fluorescent label.

102. The method of claim 101 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

103. The method of claim 100 wherein the label is a chemiluminescence label.

104. The method of claim 100 wherein the label is an electrochemiluminescence label.

105. The method of claim 100 wherein the label is biotin.

106. The method of claim 44 wherein allele specific strand displacement amplification is carried out for target nucleic acids of interest which encode a gene with two or more known alleles,

wherein the upstream and downstream ligation probe sequences are selected to be specific for a particular allele of the gene, so that the ligation probes contact, proximate the 3' end of the upstream probe or proximate the 5' end of the downstream probe, a portion of the target nucleic acid sequence which is determinative of the allele,

wherein, if the target nucleic acid does not contain the sequence determinative of the allele for which the ligation probes are selected, the juxtaposed terminus of one of the probes is

misaligned, so that, in step (b), the ligation probes are ligated only if the target nucleic acid sequence contains the allele determinative sequence, and

wherein, in step (c), amplicons are produced by strand displacement amplification if the allele determinative sequence is contained within the target nucleic acid sequence.

107. The method of claim 106 wherein the upstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the ultimate 3' nucleic acid of the upstream probe.

108. The method of claim 106 wherein the upstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the penultimate 3' nucleic acid of the upstream probe.

109. The method of claim 106 wherein the downstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the ultimate 5' nucleic acid of the downstream probe.

110. The method of claim 106 wherein the downstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the penultimate 5' nucleic acid of the downstream probe.

111. The method of claim 44 wherein the upstream and downstream oligonucleotide ligation probes are initially incapable of being ligated together, the method further comprising rendering capable of being ligated together the upstream and downstream oligonucleotide ligation probes prior to ligating the probes together in step (b).

112. The method of claim 111 wherein the rendering comprises removing one or more terminal nucleotides from the oligonucleotide probes.

113. The method of claim 112 wherein one or more terminal nucleotides are removed from the 3' terminus of the upstream oligonucleotide ligation probe.

114. The method of claim 113 wherein one or more terminal nucleotides are removed by an endonuclease.

115. The method of claim 114 wherein the endonuclease is Endonuclease IV.

116. The method of claim 112 wherein one or more terminal nucleotides are removed from the 5' terminus of the downstream oligonucleotide ligation probe.

117. A method of claim 116 wherein one or more terminal nucleotides are removed by an exonuclease.

118. The method of claim 117 wherein the exonuclease is a DNA polymerase.

119. The method of claim 112 wherein one or more terminal nucleotides that are removed are non-complementary to the target sequence.

120. A method for the amplification of at least one target nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

a. contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

b. ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

c. using the ligated target probe template in a strand displacement amplification reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, wherein each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template,

wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip.

121. The method of claim 120 wherein a target sequence is electronically addressed in step (d), before the contacting step (a).

122. The method of claim 120 wherein a ligated probe template is electronically addressed in step (d), after the ligation step (b).

123. The method of claim 120 wherein an amplicon is electronically addressed in step (d), after the amplifying step (c).

124. The method of claim 120 wherein a second SDA primer is utilized in addition to the first SDA primer in the strand displacement reaction.

125. The method of claim 120 wherein the downstream ligation probe has a 3' terminus that is modified to be non-extendable by DNA polymerases.

126. The method of claim 120 wherein a plurality of target nucleic acid sequences are amplified within the same reaction mixture.

127. The method of claim 126 wherein the first SDA primer is a universal amplification primer capable of hybridizing to all ligated target probe templates.

128. The method of claim 126 wherein at least three target nucleic acids are amplified.

129. The method of claim 126 wherein at least four target nucleic acids are amplified.

130. The method of claim 120 wherein target nucleic acids from more than one sample are amplified.

131. The method of claim 120 wherein target nucleic acids from more than two samples are amplified.

132. The method of claim 130 wherein the amplification step (c) occurs simultaneously for the target nucleic acids of more than one sample.

133. The method of claim 120 wherein no bumper primers are used in the strand displacement amplification.

134. The method of claim 120, further comprising an electronic washing step after step (d) to remove unhybridized nucleic acids which have been electronically addressed to the capture site.

135. The method of claim 120, further comprising the passing of a sufficient negative charge through the electrode associated with the capture site to create electronically induced stringency to remove mis-matched hybridized target nucleic acids formed in step (d).

136. The method of claim 124 wherein the first and second SDA primers are contained within a branched primer structure, having the capacity to support strand displacement amplification, wherein the branched primer is attached to the capture site.

137. The method of claim 136 wherein the branched primer is attached to the capture site through a biotin/streptavidin interaction.

138. The method of claim 136 wherein the branched primer is attached to the capture site through a covalent bond.

139. The method of claim 136 wherein the branched primer is formed by attaching the first and second SDA primers to an amino acid.

140. The method of claim 139 wherein the amino acid is lysine.

141. The method of claim 136 wherein the branched primer formed by attaching the first and second SDA primers to a spacer molecule selected from the group consisting of polyethylene glycol polymers, polyamino acids, and a polyfunctional amino acid which has been derivatized with two nucleic acid oligomers.

142. The method of claim 124 wherein the first and second SDA primers are individually attached to the capture site.

143. The method of claim 142 wherein the first and second SDA primers are attached to the capture site through a biotin/streptavidin interaction.

144. The method of claim 142 wherein the first and second SDA primers are attached to the capture site through a covalent bond.

145. The method of claim 120 wherein the first SDA primer is attached to the capture site.

146. The method of claim 142 wherein the first SDA primer is attached to the capture site through a biotin/streptavidin interaction.

147. The method of claim 142 wherein the first SDA primer is attached to the capture site through a covalent bond.

148. The method of claim 120 wherein at least some of the amplicon species are anchored at the capture site.

149. The method of claim 120 wherein amplicons of the ligated target probe template are detected.

150. The method of claim 149 wherein detection of amplicons is by at least one method selected from the group consisting of fluorescent detection, chemiluminescent detection, and electrochemiluminescent and detection.

151. The method of claim 149 wherein the detection is by hybridization of a labeled oligonucleotide probe to the amplicon species.

152. The method of claim 151 wherein the hybridization probe is specific for an allele of a gene.

153. The method of claim 151, further comprising the step of thermally denaturing any double stranded amplicon species prior to detecting the amplicon species.

154. The method of claim 151, further comprising the step of electronically denaturing any double stranded amplicon species prior to detecting the amplicon species.

155. The method of claim 149 wherein the amplification step and detecting step occur simultaneously in relation to one another.

156. The method of claim 149 wherein the amplification step and detecting step occur consecutively in relation to one another.

157. The method of claim 149 wherein the detection is by the incorporation of a labeled nucleotide into the amplicon.

158. The method of claim 157 wherein the amplification step and detecting step occur simultaneously in relation to one another.

159. The method of claim 120 wherein the capture site has attached thereto at least one nucleic acid selected from the group consisting of: a ligation probe, a ligation probe having a 3' terminus that is modified to be non-extendable, and a capture probe.

160. The method of claim 120 wherein the upstream and downstream oligonucleotide ligation probes are initially incapable of being ligated together, the method further comprising rendering capable of being ligated together the upstream and downstream oligonucleotide ligation probes prior to ligating the probes together in step (b).

161. The method of claim 160 wherein the rendering comprises removing one or more terminal nucleotides from the oligonucleotide probes.

162. The method of claim 161 wherein one or more terminal nucleotides are removed from the 3' terminus of the upstream oligonucleotide ligation probe.

163. The method of claim 162 wherein one or more terminal nucleotides are removed by an endonuclease.

164. The method of claim 163 wherein the endonuclease is Endonuclease IV.

165. The method of claim 161 wherein one or more terminal nucleotides are removed from the 5' terminus of the downstream oligonucleotide ligation probe.

166. A method of claim 165 wherein one or more terminal nucleotides are removed by an exonuclease.

167. The method of claim 166 wherein the exonuclease is a DNA polymerase.

168. The method of claim 161 wherein one or more terminal nucleotides that are removed are non-complementary to the target sequence.

169. The method of claim 120 wherein the restriction endonuclease which recognizes the restriction endonuclease recognition sequence is BstBNI.

170. The method of claim 120 wherein the restriction endonuclease which recognizes the restriction endonuclease recognition sequence is selected from the group consisting of: HincII, HindII, Bso BI, AvaI, Fnu4HI, Tth111I, and NciI.

171. The method of claim 120 wherein the restriction endonuclease which recognizes the restriction endonuclease recognition sequence is selected from the group consisting of: BstXI, BsmI, BsrI, BsaI, NlaV, NspI, PflMI, HphI, AlwI, FokI, AccI, TthIIII, Mra I, Mwo I, Bsr BI, Bst NI, Bst OI, and Bsm AI.

172. The method of claim 120 wherein in the strand displacement amplification step (c) at least one modified nucleic acid is incorporated into the amplicon, wherein the modification prevents cleavage of a polynucleotide containing the modified nucleic acid by restriction endonucleases.

173. The method of claim 172 wherein the modified nucleic acid is methylated.

174. The method of claim 172 wherein the modified nucleic acid is phosphorothiolated.

175. The method of claim 172 wherein the modified nucleic acid is selected from the group consisting of 2'deoxyadenosine 5'-O-(1-thiotriphosphate), 5-methyldeoxycytidine 5'-triphosphate, 2'-deoxyuridine 5'-triphosphate, and 7-deaza-2'-deoxyguanosine 5'-triphosphate.

176. The method of claim 124 wherein the strand displacement amplification in step (c) utilizes an unequal effective concentration ratio of the first SDA primer to the second SDA primer, wherein the first and second SDA primers form a set of primers, and wherein unequal populations of complementary first and second amplified strands of the target nucleic acids are formed in step (c).

177. The method of claim 176 wherein the unequal effective concentration ratio is obtained by providing at least one SDA primer of the set in molar excess as compared to the other SDA primer in the set.

178. The method of claim 177 wherein the SDA primer that is in molar excess is labeled.

179. The method of claim 178 wherein the label is a fluorescent label.

180. The method of claim 179 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

181. The method of claim 178 wherein the label is a chemiluminescence label.

182. The method of claim 178 wherein the label is an electrochemiluminescence label.

183. The method of claim 178 wherein the label is biotin.

184. The method of claim 176 wherein the unequal effective concentration ratio is obtained by providing a competitor in the amplification reaction for either the first SDA primer or the second SDA primer, and wherein the competitor is selected from the group consisting of: non-extendable competitors, non-cleavable competitors, and competitors which are non-extendable and non-cleavable.

185. The method of claim 184 wherein the competitor is anchored to a substrate.

186. The method of claim 184 wherein the competitor is in solution.

187. The method of claim 184 wherein the competitor is a non-extendable competitor.

188. The method of claim 187 wherein the competitor comprises a non-extendable 3' modification selected from the group consisting of: a 3' terminal base mis-match, a 3' dideoxy nucleic acid, and a blocking group attached to the 3' hydroxy group of the 3' terminal nucleic acid.

189. The method of claim 184 wherein the competitor is a non-cleavable competitor.

190. The method of claim 189 wherein the non-cleavable competitor comprises a nucleic acid selected from the group consisting of: methylated nucleic acids and phosphorothiolated nucleic acid.

191. The method of claim 189 wherein the non-cleavable competitor comprises a modified nucleic acid selected from the group consisting of 2'deoxyadenosine 5'-O-(1-thiotriphosphate), 5-methyldeoxycytidine 5'-triphosphate, 2'-deoxyuridine 5'-triphosphate, and 7-deaza-2'-deoxyguanosine 5'-triphosphate.

192. The method of claim 184 wherein the SDA primer which the competitor does not compete with is labeled.

193. The method of claim 192 wherein the label is a fluorescent label.

194. The method of claim 193 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

195. The method of claim 192 wherein the label is a chemiluminescence label.

196. The method of claim 192 wherein the label is an electrochemiluminescence label.

197. The method of claim 192 wherein the label is biotin.

198. The method of claim 120 wherein at least one of the first and second SDA primers is labeled.

199. The method of claim 198 wherein the label is a fluorescent label.

200. The method of claim 199 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

201. The method of claim 198 wherein the label is a chemiluminescence label.

202. The method of claim 198 wherein the label is an electrochemiluminescence label.

203. The method of claim 198 wherein the label is biotin.

204. The method of claim 120 wherein allele specific strand displacement amplification is carried out for target nucleic acids of interest which encode a gene with two or more known alleles,

wherein the upstream and downstream ligation probe sequences are selected to be specific for a particular allele of the gene, so that the ligation probes contact, proximate the 3' end of the upstream probe or proximate the 5' end of the downstream probe, a portion of the target nucleic acid sequence which is determinative of the allele,

wherein, if the target nucleic acid does not contain the sequence determinative of the allele for which the ligation probes are selected, the juxtaposed terminus of one of the probes is misaligned, so that, in step (b), the ligation probes are ligated only if the target nucleic acid sequence contains the allele determinative sequence, and

wherein, in step (c), amplicons are produced by strand displacement amplification if the allele determinative sequence is contained within the target nucleic acid sequence.

205. The method of claim 204 wherein the gene has two known alleles, and the target nucleic acids are addressed to two capture sites.

206. The method of claim 204 wherein the gene has multiple known alleles, and the target nucleic acids are addressed to multiple capture sites.

207. The method of claim 204 wherein the upstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the ultimate 3' nucleic acid of the upstream probe.

208. The method of claim 204 wherein the upstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the penultimate 3' nucleic

acid of the upstream probe.

209. The method of claim 204 wherein the downstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the ultimate 5' nucleic acid of the downstream probe.

210. The method of claim 204 wherein the downstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the penultimate 5' nucleic acid of the downstream probe.

REMARKS

Applicants file herewith a divisional application of parent applications USSN 09/290,577, which will issue as U.S. Patent No. 6,238,868 on May 29, 2001. Applicants note that the amended claim set contains two independent claims 44 and 120, and a more expansive set of dependent claims than filed in the previous parent application. Applicants submit that these dependant claims are clearly supported by the original specification as filed (including the original claims), but invite the Examiner to contact the undersigned if she has any questions regarding the location of support for any particular dependant claim.

Applicants also wish to note that independent claim 44 recites that steps a, b, and c occur at the same time. By this phrase, applicants mean that at some point during the amplification process, steps a (hybridization of the ligation probes to the target nucleic acid), b (ligation of the ligation probes into the ligation probe template) and c (SDA of the ligation probe template) occur simultaneously in the reaction mixture by the concerted action of the ligase and the SDA enzymes in the reaction mixture upon various molecules of the same species (e.g., two copies of the same

mRNA target nucleic acid.) However, step a may still occur prior to that point in time, or step c may occur after that point in time.

Applicants also note that step c recites the use of at least one SDA primer in the SDA reaction. The general enzymatic mechanism of the ligation-dependant SDA reaction is shown in Figure 23. As is self-evident from Figure 23 A and B, the utilization of a first SDA primer leads to the linear amplification of the target DNA sequence in two ways. First, the target strand is displaced in the formation of product I, allowing the further hybridization and ligation of the ligation probes (feeding back into the top of figure 23 A). Second, the strand displacement amplification reaction depicted in figure 23 B also creates a feedback loop which linearly amplifies the target nucleic acid sequence. Thus, the use of a first SDA primer for SDA amplification effectively amplifies the target nucleic acid sequence. In addition, one may utilize a second SDA amplification primer, as shown in figure 23 C, to further amplify the target nucleic acid.

Applicants request prompt consideration of the divisional application. If the Examiner has any questions regarding the application, she is invited to contact the undersigned at (949) 567-2305.

Respectfully submitted,

LYON & LYON LLP

Dated: May 25, 2001 By: _____
Patrick S. Eagleman, Reg. No. 44,665

633 West Fifth Street, Suite 4700
Los Angeles, California 90071-2066
949/567-2300 X 1105 Telephone
213/955-0440 Facsimile